1: J Immunol 1991 Jun 15;146(12):4414-20 Related Articles, Books, LinkOut

T cells from tumor-immune mice nonspecifically expanded in vitro with anti-CD3 plus IL-2 retain specific function in vitro and can eradicate disseminated leukemia in vivo.

Crossland KD, Lee VK, Chen W, Riddell SR, Greenberg PD, Cheever MA.

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The therapeutic efficacy of adoptive immunotherapy of cancer has been shown to positively correlate with the dose of tumor-immune T cells transferred. Therefore, the success of this therapy is critically dependent on the ability to procure large numbers of functionally active T cells. Previous studies in animal models have shown that the limited therapeutic efficacy of a small number of immune T cells can be greatly enhanced by expansion of T cells in vitro to greater numbers before transfer in vivo. Optimal regimens for T cell expansion in vitro have generally employed the use of intermittent stimulation of the TCR with specific Ag followed by exogenous IL-2. The use of IL-2 alone does not provide for requisite episodic up-regulation of IL-2R. Stimulation of the invariant CD3 portion of the TCR/CD3 complex with antibody to CD3 (anti-CD3) represents an alternative method of up-regulating IL-2R and has been used to nonspecifically induce the growth of Ag-specific T cell lines and clones long-term in vitro with maintenance of function and specificity. The current study examined whether resting T cell populations containing small numbers of memory tumor-specific T cells could be rendered more effective in tumor therapy by nonspecific expansion in vitro with anti-CD3 plus IL-2. Spleens from C57BL/6 mice previously immunized to FBL-3, a syngeneic virus-induced leukemia, were nonspecifically stimulated with anti-CD3 plus IL-2. The resultant T cells were expanded in number, were nonlytic to FBL-3 but retained the ability to become lytic upon specific stimulation by FBL-3, and were effective in specific tumor therapy. The Ag-specific anti-tumor immune function declined on a per cell basis after each cycle of anti-CD3-induced T cell expansion. However, the approach resulted in a substantial increase in total T cell number and an overall net increase in the function of the effector T cell population. Thus, stimulation of tumor-immune T cell populations with anti-CD3 plus IL-2 represents a nonspecific method for expanding the number of specific effector T cells for cancer therapy.

PMID: 1674958 [PubMed - indexed for MEDLINE]

1: Int Immunol 1991 Mar;3(3):255-64 Related Articles, Books, LinkOut

High-frequency activation of single CD4+ and CD8+ T cells to proliferate and secrete cytokines using anti-receptor antibodies and IL-2(1).

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A high cloning efficiency single-cell culture system was developed to define the activation requirements of isolated CD4+ and CD8+ T cells to proliferate and secrete cytokines. T cells were triggered using solid-phase anti-CD3 and anti-CD4 or anti-CD8 antibodies plus rIL-2. Activation was measured by microscopic scoring of proliferation and by measurement of cytokine production using the cytokine-responsive cell lines FDC-P1, which responds to GM-CSF, IL-3, IFN-gamma and IL-4, and 32D clone 3 which responds to IL-3 only. Whilst anti-CD3 plus rIL-2 triggered only 4% of peripheral T cells to proliferate, anti-CD3 plus anti-CD8 mAb triggered about 40% of CD8+ T cells; 80% of the resultant clones secreted cytokine and 90% of these were IL-3+. Anti-CD3 plus anti-CD4 mAb triggered proliferation in about 20% of CD4+ T cells, of which 34% formed cytokine-producing clones with 47% of these secreting IL-3. In addition to responding at higher frequency, CD8+ T cells formed larger clones which produced higher levels of cytokines than CD4+ cells. Cell separation on the basis of Pgp-1 expression suggested that this culture system did not select for previously activated cells. Whereas Pgp-1+ T cells from keyhole limpet haemocyanin (KLH)-primed mice were enriched in KLH-specific cells, no significant differences were observed in the clonogenicity or cytokine-secreting capacity of Pgp-1+ and Pgp-1- T cells from normal mice.

PMID: 1675587 [PubMed - indexed for MEDLINE]

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1: Exp Hematol 2002 Mar;30(3):245-51 Related Articles, Books, LinkOut

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Ex vivo expansion, maturation, and activation of umbilical cord blood-derived T lymphocytes with IL-2, IL-12, anti-CD3, and IL-7. Potential for adoptive cellular immunotherapy post-umbilical cord blood transplantation.

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OBJECTIVES: We investigated whether umbilical cord blood (UCB) T cells could be ex vivo expanded and activated in short-term culture for potential utilization as adoptive cellular immunotherapy post-umbilical cord blood transplantation (UCBT). METHODS: Fresh UCB mononuclear cells (MNCs) were isolated by Ficoll density centrifugation. Cryopreserved UCB mononuclear cells were thawed and washed with 2.5% human serum albumin and 5% dextrose in isotonic saline. The nonadherent MNC fraction were then plated in a serum-free cocktail of IL-2, IL-12, and anti-CD3 with and without IL-7 for 48 hours. Proliferation, cytotoxicity, TH1 (IFN-gamma), CD25, and CD45RO assays were performed. RESULTS: Proliferation studies demonstrated a significant increase in the proliferative ability of the UCB MNCs incubated in anti-CD3, IL-2, IL-12, and IL-7 (fresh--$p < 0.005$, and thawed--$p < 0.001$). The combination of all four agonists significantly induced expression of CD45 RO (fresh--$p < 0.05$, and thawed--$p < 0.001$) in both the CD4(+) and CD8(+) T cells expressing CD25 (fresh UCB--$p < 0.01$ [CD4] and $p < 0.005$ [CD8], respectively; thawed UCB--$p < 0.001$ [CD4] and $p < 0.001$ [CD8]). Intracellular cytokine profiles also revealed a significant increase in the production of IFN-gamma (TH1 cells) (fresh UCB--$p < 0.005$, and thawed UCB--$p < 0.001$). The combination also significantly increased the killing of K562-labeled target cells (fresh--$p < 0.0001$, and thawed--0.731 +/- 0.03 vs 0.16 +/- 0.01) ($p < 0.001$). CONCLUSIONS: These data suggest that the ex vivo combination of IL-2, IL-12, anti-CD3, and IL-7 significantly enhances the proliferation, activation, maturation, and cytotoxic potential of UCB T cells of both fresh and thawed UCB MNC. Further studies, however, are required to determine whether these ex vivo--expanded MNC could also potentially exacerbate acute or chronic graft-vs-host disease and/or other toxicities if utilized post-UCBT.

1: J Immunol Methods 2002 Apr 1;262(1-2):71-83 Related Articles, Books, LinkOut

Ex vivo expansion of functional T lymphocytes from HIV-infected individuals.

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This study was designed to define the conditions for expansion of functional T lymphocytes from human immunodeficiency virus (HIV)-infected subjects, with the ultimate goal of using these cells for immunotherapy. The most appropriate culture conditions for good T cell proliferation included stimulation with anti-CD3 and anti-CD28 coated microspheres, and propagation in Aim V serum-free media with 20 U/ml interleukin-2 (IL-2), supplemented with decreasing concentrations of serum for the initial 8 days. Under these conditions, a 14-day culture period yielded approximately a 10,000-fold expansion of T lymphocytes from HIV-infected donors. The cultured cells comprised approximately 15% CD4(+) cells and 70% CD8(+) cells. These cells retained functional capacity as assessed by cytotoxicity towards HIV proteins, and production of IL-2 and interferon-gamma (IFN-gamma). Viral replication within the culture system was controlled, but not eliminated, without the requirement for antiviral agents. These culture conditions were demonstrated to be suitable for larger scale expansion of cells in hollow fibre bioreactors. This methodology provides a suitable means of producing large quantities of functional T cells for use in autologous immunotherapy protocols.

PMID: 11983220 [PubMed - in process]

1: Cell Immunol 1984 Sep;87(2):473-84

Influence of age on the ability of thymic adherent cells to produce factors in vitro which modulate immune responses of thymocytes.

Sato K, Chang MP, Makinodan T.

Thymic adherent cells from BALB/c mice ranging in age from 1 day to 20 months were cultured in vitro for 1 month. The supernatants, collected at weekly intervals, were assessed for their ability to augment the antigen/mitogen responses of thymocytes from 2- or 4-week-old BALB/c mice and spleen cells from 3-month-old nude BALB/c mice. The results indicate that (a) the ability of thymic adherent cells to produce an augmenting factor(s) declines sharply between 2.5 and 5 months of age; (b) thymic adherent cells of 1-day-old mice synthesize an inhibitory factor(s) in addition to the augmenting factor, while those of young adult mice synthesize only the augmenting factor, and those of 20-month-old mice synthesize primarily the inhibitory factor; (c) supernatants containing the augmenting factor can be neutralized by mixing them with supernatants containing the inhibitory factor; (d) thymocytes which are responsive to the augmenting factor are immature as judged by their sensitivity to dexamethasone and by their ability to bind macrophages; and (e) spleen cells of normal and nude mice are not responsive to either the augmenting or the inhibitory factor of the supernatant.

PMID: 6331895 [PubMed - indexed for MEDLINE]

1: J Immunol 1990 Apr 15;144(8):2875-82 Related Articles, Books, LinkOut

Anti-CD3 antibodies induce T cells from unprimed animals to secrete IL-4 both in vitro and in vivo.

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Recently, functional heterogeneity among Th cells has been recognized. Based on pattern of lymphokine secretion, two mutually exclusive subsets of CD4+ cells have been defined and designated Th1 (secreting IL-2 and IFN-gamma) and Th2 (secreting IL-4 and IL-5). Identification of these subsets was mostly based on the study of long term cultured T cell lines and clones, and little is known about the Th heterogeneity in vivo. In particular, it has been suggested that IL-4 producing cells cannot be detected in vivo or in primary stimulations in vitro unless responder cells had been previously primed. Our data however, indicate that anti-CD3 mediated stimulation can induce T cells isolated from unprimed animals to IL-4 production. An assay system based on the ability of IL-4 to increase Ia expression of B cells present in the environment of activated T cells was found to be more sensitive than detection of secreted IL-4 in the supernatant by conventional bioassays and was used to study IL-4 production by unprimed lymphocytes polyclonally stimulated in vivo and in vitro by anti-CD3 mAb. The results obtained indicate that CD4+ CD8- T cells able to produce IL-4 upon receptor-specific stimulation exist in the preimmune pool of adult animals. Remarkably, these cells can also be stimulated in vivo by treating animals with anti-CD3 mAb, as indicated by the in vivo induction of IL-4 specific mRNA and hyper-Ia expression on B cells. These results indicate that the inability to detect IL-4 in primary cultures is not due to different activation requirements of Th2 cells but may simply result from their lower frequency in unprimed animals.

PMID: 1969874 [PubMed - indexed for MEDLINE]

1: J Immunol 1996 Mar 15;156(6):2062-7 Related Articles, Books, LinkOut

Uncoupling IL-2 production from apoptosis and TNF production by changing the signal through the TCR.

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T cells may discriminate between stimuli in a variety of ways, including the presence of cytokines or other costimulatory signals, the type of Ag (peptide, superantigen, or allorecognition), or the magnitude of the signal through the TCR. We have used anti-CD3 stimulation of T hybridomas to examine signals generated through the TCR in the absence of exogenous APCs. Soluble whole anti-CD3, but not F(ab')2 anti-CD3, was able to stimulate the T hybridomas to produce IL-2. Plastic-bound anti-CD3, in contrast, stimulated TNF production, G1 arrest, and apoptosis by the T hybridoma. Engagement of the CD4 coreceptor on these cells had no effect on the overall pattern of signaling observed. Although TNF production was correlated with apoptosis, anti-TNF treatment did not prevent cell death or G1 arrest. The response of the T hybridoma to both forms of anti-CD3 included significant IL-2 production even at the lowest dose tested. However, soluble anti-CD3 at the highest dose tested elicited only minor apoptosis, while plastic-bound anti-CD3 elicited significant apoptosis even at the lowest dose. The difference in response was not evident at the level of phosphotyrosine proteins two min after cross-linking of the TCR.

PMID: 8690893 [PubMed - indexed for MEDLINE]

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Altered cytokine production and accessory cell function after HIV-1 infection.

Yoo J, Chen H, Kraus T, Hirsch D, Polyak S, George I, Sperber K.

Division of Clinical Immunology, Mount Sinai Medical Center, New York, NY 10029, USA.

We investigated cytokine production and accessory cell function in human macrophage hybridoma cell lines and primary monocytes after infection with HIV-1. HIV-1 infection induced IL-10 production in the macrophage hybridoma cell line with loss of IL-12 1 wk after infection. There were also significant increases in production of IL-10 (537 +/- 521 vs 687 +/- 625 pg/ml) while there was a reduction in IL-12 (6.3 +/- 3.1 vs 1.2 +/- 1.0 pg/ml, p = 0.021) in the primary monocytes 5 days after HIV-1 infection. In addition, the hybridoma cell lines and primary monocytes failed to support PHA, Con A, PWM, or anti-CD3- induced T cell proliferation 1 wk after infection. The viability of the T cells cocultured with the HIV-1-infected macrophage cell lines or the primary monocytes as determined by propidium iodide staining was unaltered and there was no increase in apoptosis-specific DNA strand breaks or increased expression of Bcl-2 in the T cells. No soluble suppressor factor was present, since UV-inactivated supernatants from the hybridoma cell line and primary monocytes failed to inhibit mitogen- and anti-CD3-induced T cell proliferation. Early events in T cell activation, including calcium flux and phosphotyrosine kinase activity, were intact in the T cells cocultured with the HIV-1- infected hybridomas and monocytes but there was reduced IL-2 production. Addition of exogenous IL-2 restored the proliferative responses. Taken together, these data suggest that alteration of cytokine production and accessory cell function for mitogens and anti-CD3-induced T cell proliferation independent of induction of apoptosis, suppressor factor production, or inhibition of T cell signaling occurs very early after HIV-1 infection and may contribute to the global immunosuppression observed in AIDS.

PMID: 8757640 [PubMed - indexed for MEDLINE]

1: Cancer Immunol Immunother 1998 Jul;46(5):268-76 Related Articles, Books, LinkOut

Ex vivo activation of tumor-draining lymph node T cells reverses defects in signal transduction molecules.

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The adoptive transfer of tumor-draining lymph node (LN) T cells activated ex vivo with anti-CD3 and interleukin 2 (IL-2) mediates the regression of the poorly immunogenic murine melanoma D5. The efficacy of the activated LN cells is augmented when the sensitizing tumor is a genetically modified variant (designated D5G6) that secretes granulocyte/macrophage-colony-stimulating factor. In contrast to anti-CD3/IL-2-activated LN cells, adoptive transfer of freshly isolated tumor-draining LN T cells has no therapeutic activity. To determine whether the acquisition of antitumor function during ex vivo activation is associated with modifications in signal transduction capacity, the protein tyrosine kinases p56lck and p59fyn and proteins of the NF-kappaB family were analyzed in tumor-draining LN T cells. The levels of p56lck and p59fyn were lower in tumor-draining than in normal LN T cells and production of tyrosine-phosphorylated substrates was markedly depressed following anti-CD3 stimulation. After 5-day anti-CD3/IL-2 activation, levels of p56lck and p59fyn and protein tyrosine kinase activity increased. Interestingly, the levels of p56lck, p59fyn, and tyrosine kinase activity were higher in activated T cells derived from LN that drained D5G6 than they were in those from D5 tumors. In contrast, the cytoplasmic levels of c-Rel and Rel A were normal in freshly isolated tumor-draining LN, as was nuclear kappaB DNA-binding activity induced by anti-CD3 mAb or phorbol myristate acetate. Stimulation of activated LN cells with D5 tumor cells induced the nuclear translocation of NF-kappaB. These findings indicate that the recovery of proteins mediating signal transduction through the T cell receptor/CD3 complex in LN T cells activated ex vivo was associated with the acquisition of antitumor function.

PMID: 9690455 [PubMed - indexed for MEDLINE]

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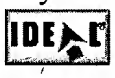

High serum levels of soluble IL-2 receptor, cytokines, and C reactive protein correlate with impairment of T cell response in patients with advanced epithelial ovarian cancer.

Maccio A, Lai P, Santona MC, Pagliara L, Melis GB, Mantovani G.

Department of Medical Oncology, University of Cagliari, Italy.

The serum levels of interleukin-(IL-)1 alpha, IL-1 beta, IL-2, IL-6, TNF alpha, and sIL-2R and the proliferative response of peripheral blood mononuclear cells (PBMC) to phytohemagglutinin (PHA), anti-CD3 monoclonal antibody (mAb), recombinant IL-2 (rIL-2), and the combination of PHA or anti-CD3 mAb with rIL-2 were studied and correlated with serum levels of C-reactive protein (CRP) in women with advanced epithelial ovarian cancer. The expression of CD25 and CD122 subunities of membrane-bound IL-2R on PHA- or anti-CD3 mAb-stimulated PBMC was also studied. In comparisons with the controls, PBMC response to PHA, anti-CD3 mAb, and rIL-2 was significantly lower in the cancer patients. The addition of exogenous rIL-2 to the PBMC cultures increased response in both controls and patients but did not modify the significance of the differences. After stimulation with PHA or anti-CD3 mAb, the percentage of PBMC CD25+ or CD122+ was significantly lower in patients. The serum levels of IL-1 alpha, IL-1 beta, IL-6, TNF alpha, sIL-2R, and CRP were significantly increased in patients compared to the controls. Instead, no differences were observed for serum levels of IL-2. A strong association was found between high serum levels of the above-mentioned cytokines, sIL-2R, and CRP. The results of our study on advanced stage (IIIb-IV) ovarian cancer patients are consistent with the previously reported hypothesis that high IL-6 and/or CRP serum levels may represent an important and independent prognostic factor of the likely outcome in cancer patients.

PMID: 9648596 [PubMed - indexed for MEDLINE]